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The Isolation of *Streptomyces species* in Different Soil Sources from Middle Anatolian Regions of Turkey

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Abstract: *Streptomyces* is the largest species of the *actinomycetes* group, with more than 500 defined species, aerobic, gram-positive, and phylogenetic class filamentous (thin protruding in thread form). It is a large group that is mostly found in the soil and as a secondary metabolite of its fermentation, it enables the production of various and important components (antibiotics, chemotherapeutics, fungicides, herbicides, and immunosuppressants) in the field of industry and medicine. In this study, six bacterial isolates were isolated from soil samples in different regions of Turkey. Morphological characteristics, gram staining, and PCR test were applied for identification. Six isolates, *Streptomyces mutabilis, S. collinus, S. peucetius, S. cyaneofuscatus, S. albogriseolus* and, *S. griseoflavus,* were compared with the general characteristics of the *Streptomyces* species in International *Streptomyces* Project. Air and reverse side mycelium color were determined, and all were confirmed by the gram-positive test. Studies have shown that the regions of Ankara and Konya are rich in *Streptomyces* species.

Keywords: *Actinomycetes*; *Streptomyces cp.*; Isolation; Antibiotic; Secondary metabolite.

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INTRODUCTION

Actinomycetes are bacteria belonging to the *Actinomycetales* group known as *actinobacteria*. They are spore-forming gram-positive bacteria and are characterized by the formation of mycelium, which protrudes on the medium and produces asexual spores (1). Compared to yeast and other microorganisms, they contain high amounts of guanine and cytosine in their DNA structure (2). Within 24 hours, colonies that can only be seen under a microscope are formed. Colony formation is very slow. It takes 3-4 days to be seen with the naked eye. It takes 7-14 days of incubation to see mature mycelium. *Streptomyces avermitilis* is a spore-forming, aerobic gram-positive bacteria belonging to the mesophilic actinomycete group. Spores are spherical or oval and usually occur in chains. Albumin, glycerol-asparagine, inorganic salt, starch, and oatmeal agar media are required for sporulation to occur. Gray spores form on light brown agar and the colony reverses from dark brown to tan. Culture grows best between 27-37 °C. This group has a strong tendency to produce antibiotics with different chemical structures and biological activities (3).

The presence and distribution of *Streptomyces* species belonging to *actinomycetes*, the most abundant group in soil, are highly affected by the physical and chemical conditions of the soil such as temperature, pH, types of organic materials, and moisture content. Acidic-resistant groups are the most abundant *actinomycetes* in acidic soil, while they are less abundant in soils with alkaline pH (4). They are widely recognized for their ability to produce industrially important enzymes, and secondary metabolites during fermentation, and, in addition, to cover about 80% of antibiotic products (5). The identification and isolation of

microorganisms producing secondary metabolites have been the focus of research for several years (6).

Chemical, molecular and taxonomic properties of the genus *Streptomyces* have been revealed by several studies (7–12). According to this, non-acidic, rarely fragmented submerged hyphae (0.5-2 µm) are organisms in which reproduction occurs by dormant spores at the ends of the aerial hyphae (13). A few exceptional species form short spore chains in submerged mycelium; *sclerotium, pycnidium*, *sporangium,* and synnemata-like structures can be formed (14). Colonies at the beginning of growth are smooth and soft, then hard-tight, cottony, granular, powdery, or velvety. Most strains can produce species-specific antibiotics (3). A large number of pigments responsible for the color of the submerged micelle can be self-produced, as well as secreted pigments.

The cell wall contains large amounts of L-Diaminopimelic acid (L-DAP) in its peptidoglycan structure. It contains no mycolic acid and saturated iso and anteiso fatty acids, hexa and octa dehydrogenated menaquinone with nine isoprene units as the predominant isoprenoid. It also contains complex polar oil containing phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol with these oil characteristics, it belongs to the phospholipid type 2 group. The Guanine+Cytosine ratio of its DNA varies between 69-78%. It is mostly rotten; a few species are rarely pathogenic in humans and animals, some in plants. The number of species in the genus Streptomyces is increasing (15).

More than 650 species were reported in the German Collection of Microorganisms and Cell Cultures (DSMZ). Thus, this genus became a member of the genus *Actinobacteria* with the highest number of species in the order *Actinomycetales* (16). Based on all these classifications, *Streptomyces* has been divided into 20 major, 41 minor, and 22 single-member groups. Large classes are considered to be the groups of species consisting of six or more types of strains, while small classes are considered to be a single species consisting of 2-5 types of strains (16–20).

Contents of the medium affect *Streptomyces* isolation (5). It has been observed that the best isolation occurs in media containing glycerol or starch as a carbon source, and arginine, casein, or nitrate as a nitrogen source. Different antifungal agents called nystatin, cycloheximide, and pimaric are generally used during isolation to obtain pure bacterial isolates. *Streptomyces* are identified by their spore size, morphology, chains, pigmentation, physiological and biochemical properties, and resistance to antibiotic resistance (21).

Standard microbiological methods, biochemical analysis, and DNA sequencing are used to selectively identify the genus and species of isolated microorganisms. *Streptomyces* forms stable filaments and can produce long-chain spores that grow in aerobic conditions. Direct and indirect screening methods to determine antibiotic producers have often been used to isolate a particular microorganism. While direct screening of strains includes bioassay or chemical methods, indirect screening includes the correlation of antibiotic production with strain characteristics (11). *Streptomyces* members are very important due to their ability to produce various types of secondary metabolites such as Valinomycin, Neomycin B, Avermectin, Neomycin C, Bicosamycin, Colabomycin A, Colabomycin C, Germakradionel, Hormaomycin, etc. (5).

Isolation and identification of *Streptomyces cp*. were carried out by three basic methods in the literature (22–24). Siddique et al (2014) performed isolation by using different types of media such as *Actinomiycete* isolation medium (AC) and Kuster's isolation medium (KU) for the single colony, Yeast Extract Malt Extract Glucose Medium (YMG) and *Streptomyces* isolation medium (SC) for preculture and culture growths (22). Ariffuzzaman et al (2010) tested Glycerolarginine isolation medium (GAM) for the single colony, Modified starch casein agar medium (MSCAM), Tryptone-soybean agar medium (TSA) and Yeast Extract Malt Extract Glucose Medium (YMG) for the preculture and culture growths (23). Kumar et al (2010) determined the isolation of *Streptomyces cp.* by starch casein agar (SCAM) for a single colony and Yeast Extract Malt Extract Glucose Medium (YMG) for the preculture and culture growths (24).

In this study, *Streptomyces* antibiotic-producing bacteria were isolated from soil samples from Ankara and Konya regions. Different media were used to screen pure *Streptomyces* strains. The antibacterial activities of various isolates were evaluated, and their morphological structures were determined.

MATERIALS AND METHODS

Isolation and Identification of *Streptomyces* from Soil

The soil suspended in sterile water is diluted and spread on selective agar medium and then subjected to aerobic incubation at 25-28 °C.

Collection and Preparation of Soil Samples

Soil samples were taken from Ankara and Konya regions. The methods used in sampling and preparation are given below.

Method 1 (22)

Soil samples are taken with a sterile spatula by digging 3 cm from the soil surface and stored in

clean, dry and sterilized polyethylene bags at 40 °C until pre-treatment. Each 1-g soil sample is suspended in 100 mL of sterile 0.9% NaCl solution and incubated at 28°C for 30 min at 180 rpm in an orbital shaker. Samples are spread on petri dishes containing *Actinomiycete* isolation medium (AC), (pH=7, 5 g/L glycerol, 2 g/L KH_2PO_4, 0.1 g/L asparagine, 0.1 g/L $MgSO_4.7H_2O$, 1 mg/L $FeSO_4.7H_2O$, and 15 g/L agar) and Kuster's isolation medium (KU), (pH=7.1, 10 g/L soluble starch, 2 g/L KNO_3, 2 g/L K_2HPO_4, 2 g/L NaCl, 0.05 g/L $MgSO_4.7H_2O$, 0.02 g/L $CaCO_3$, 0.01 g/L $FeSO_4.7H_2O$, and 18 g/L agar) and incubated for 7-10 days at 28 °C.

Samples grown on *Actinomycete* isolation (AC) and Kuster's agar media (KU) were incubated while the ones that could be *Streptomyces* were labeled, and single colony screenings were made on Yeast Extract Malt Extract Glucose Medium (YMG) (pH=7.3, 4 g/L yeast extract, 10 g/L malt extract, 4 g/L glucose, 20 g/L agar) containing 0.05 mg/mL nystatin and incubated at 28 °C for 7-10 days for colony formation.

Single colonies are transferred to *Streptomyces* isolation medium (SC) (pH= 7.0) consists of 5 g/L glucose, 4 g/L L-glutamic, 19 g/L K_2HPO_4, 0.7 g/L $MgSO_4.7H_2O$, 1 g/L NaCl, 3 mg/L $FeSO_4.7H_2O$, 25 g/L agar, and 0.05 mg/L nystatin.

Method 2 (23)
Soil samples are taken with a sterile spatula by digging 4 cm from the soil surface and stored in clean, dry and sterilized polyethylene bags at 40 °C. Each 1-g soil sample is diluted 1:10 and 1:100 with sterile water or 0.9% NaCl solution, and the soil suspensions are heated in a water bath at 50 °C for 10 minutes. Samples are spread on petri dishes containing Glycerolarginine (GAM) isolation medium (pH 7.4) consists of 1 g/L L-Asparagine, 1 g/L Dipotassium phosphate, 0.01 g/L $FeSO_4.7H_2O$, 0.001 g/L $MgCl_2.4H_2O$, 0.001 g/L $ZnSO_4$. $7H_2O$, 20 g/L Agar, and 0.05 mg/L Nystatin and incubated at 25 °C for 7-10 days.

While the soil samples growing in GAM medium are in the incubation stage, colonies that could be *Streptomyces* are marked. Single colony was cultivated on modified starch casein agar medium and incubated for colony formation at 28 °C for 7-10 days. Modified starch casein agar medium (MSCAM) (pH=7.2) consists of 10 g/L soluble starch, 15 g/L agar, 50% sea water, and 0.05 mg/mL nystatin. Cultures taken from this isolation medium are plated on Modified starch-casein agar medium to which cyclohexamide and nystatin (0.05 mg/mL) have been added. The formed colonies are transferred back to Tryptone-soybean agar medium. Colonies are observed on this medium by incubating at 25 °C for 2-7 days. Tryptone-soybean agar medium (TSA) (pH=7): 15 g/L peptone from casein, 5 g/L peptone from soybean meal, 5 g/L NaCl, and 15 g/L Agar.

Cultures showing growth in TSA medium are transferred to YMG medium, and the strains are maintained at 4 °C for 2-month periods and serially maintained for a longer period. It is considered a *Streptomyces* culture if it grows in a YMG medium.

Method 3 (24)
Soil samples are taken from a depth of 10-15 cm. The samples are air-dried for 1 week, crushed, and sieved. Each 1-g soil sample is suspended in 100 mL of sterile 0.9% NaCl and incubated at 28 °C in an orbital shaker for 30 minutes at 180 rpm. Samples are spread by taking 0.1 mL into petri dishes containing starch casein agar (SCAM) isolation medium and incubated at 28-30 °C and monitored for 48, 72, and 96 hours. SCAM (pH 7.2) consists of 10 g/L soluble starch, 1 g/L casein powder 50% seawater, and 15 g/L agar. While the soil samples grown in the SCAM medium are in the incubation stage, bacteria that may be *Streptomyces* species are marked and a single colony is cultivated in the SCAM medium and colony formation is observed at 28 °C for 7-10 days. Repetitive transfers of cultures from SCAM isolation media are performed on YMG media. Maintained at 4 °C after repeated transfers. Selected samples are cultivated in a single colony in YMG medium.

Morphological Characterization
The morphology of *Streptomyces* colonies after colony proliferation was characterized based on air mycelial morphology, and reverse side color principles. Microscopic slides were prepared for all isolates and examined under the microscope.

Gram Stain
Gram-positive responses were investigated for *Streptomyces* isolates. A small amount of distilled water is dripped onto the coverslip in the sterile cabinet, and a single colony is taken from the petri dish with the help of a loop and spread homogeneously on the coverslip. The water in the lamella is evaporated on the burner flame. Crystal violet solution is dripped onto the coverslip and left for 1 minute. After a 1-minute period, the excess crystal violet solution is disposed of. Lugol solution is dripped and left for 1 minute. The preparation is washed with distilled water. Excess water on the lamella is disposed of. It is washed with a decolorizing solution and then washed with distilled water. It is dyed with saffron, left for 1 minute. The preparation is washed with plenty of distilled water and dried. Some immersion oil is poured onto the dried preparation. It is examined under a microscope.

Microorganisms seen in purple are marked as gram (+), and those seen in pink-red color are marked as grams (-).

DNA Isolation, Polymerase Chain Reaction (PCR), and Sequence Analysis

YMG liquid nutrient medium is prepared; the selected samples are mixed with the aid of the loop and microorganism proliferation is observed. At the end of the process, 2 mL of the sample taken from the YMG liquid medium was used for DNA isolation. For isolation GeneJET Genomic DNA Purification Kit (Thermo Cat No: K0721) was used and the following steps are applied in order. 2 mL of liquid YMG medium with microorganisms is taken into Eppendorf tubes with the help of a micropipette then sample is centrifuged at 5000 g for 10 min. The supernatant is disposed of, and the precipitate is centrifuged once again under the same conditions. The supernatant is disposed of without disturbing the pellet. In order to lyse the cells, 200 µL of Lysis Buffer (20 mM Tris-HCl, pH:8, 2 mM EDTA, 1.2% TritonX-100, and 20 mg/mL lysozyme) is added and incubated for 45 min at 37 °C. 10 µL of Lysozyme enzyme is added and waited for a minute. At the end of the incubation, 200 µL of lysis solution and 100 µL of Proteinase K are added and mixed thoroughly and incubated for 30 min at 56 ^{0}C. 20 µL of RNase A is added to the solution and incubated for 10 min at room temperature. 400 µL of 50% ethanol is added to the solution and the suspension is transferred to the column with mixing. After spinning down at 6000 g for a minute, the bottom part is discarded, and the column is transferred to new tubes. 500 µL of Washing Solution I is added and centrifuged at 8000 g for a minute. The bottom liquid is discarded, and 500 µL of Washing Solution II is added to the column and centrifuged at maximum speed for 3 minutes. The bottom liquid is discarded, and the column is centrifuged again for 1 min at maximum speed. After centrifugation, the column is transferred to a new 1.5 mL microcentrifuge tube and 50 µL of elution buffer is added. DNA samples are stored at -20 °C after centrifugation at 8000 g for a minute.

The primers, which came in lyophilized form, were first diluted with ultrapure water to 100 µmol per microliter. The stock is diluted to 10 µmol per microliter for later use in PCR. PCR is performed using DreamTaq DNA Polymerase (Cat: EP0703). Standard 3-step PCR Cycling is as follows, initial denaturation for a cycle for 5 min at 95 °C, denaturation for 35 cycles for 30 sec at 95 °C, 30 sec at 58 °C and a min at 72 °C, final extension for a cycle for 10 min at 72 °C.

~920 base pair PCR amplicons are submitted for Forward-Reverse DNA sequencing specific to the 16S rDNA gene. The nucleotide sequences resulting from the sequencing reaction are aligned to the NCBI database with the "Basic Local Alignment Search Tool (BLAST)" algorithm.

The obtained genomic DNA is tested in RAPD reactions to show the suitability of the isolated DNA for PCR reactions. Electrophoresis of DNA samples is performed on gels containing 1% agarose. After PCR, 5 µL of the obtained products are taken, mixed with 1 µL of 6X loading paint and run under 100-Volt electric current. The 1 kb DNA Marker (Fermentas, Finland) is used to determine the size of the approximately 920 bp long amplicon. After electrophoresis, the gels are stained in 0.2 µg/mL ethidium bromide (Biotium, Cat: 40042) solution for 20 minutes and visualized and photographed under UV light at 366 nm wavelength using a transilluminator.

Antimicrobial Activity with Well Management

An antimicrobial activity study was planned on the determined species, and according to this study, *Escherichia coli* (*E .coli*) and *Staphylococcus* (*Staph*) pathogenic microorganisms were selected and the effectiveness of antibiotic production of microorganisms on these pathogens was examined.

Lysogeny broth (LB) solid, LB liquid and LB soft media were prepared and sterilized. 5 µL of *E. coli* and *Staph* were separately inoculated in 5 mL of LB liquid medium. Pathogens were activated by incubation at 37 °C at 180 rpm for 18 hours. LB solid medium was prepared in glass tubes as 5 mL and LB solid medium was prepared in petri dishes as 10 mL. At the end of 18 hours, 1 µL of activated pathogens was taken and inoculated into 5-mL LB soft tubes after the media was melted at 95 °C and mixed well, then poured onto LB solid petri dishes. After media solidifies, wells were drilled in the divided areas of the petri dish with the well drilling apparatus. Two of the wells were filled directly by taking samples from the liquid fermentation media of isolated microorganisms, and two of them were filled with centrifugated sample. One well was filled with physiological saline for negative control purposes. After 10-15 minutes of waiting period, petri dish was left to incubate for 18 hours, and at the end of the incubation antimicrobial activity was observed.

RESULTS AND DISCUSSION

The isolation of *Streptomyces* type antibiotic-producing bacteria was carried out from six soil samples in Ankara and Konya regions by using 3 different nutrient media. After 5-7 days of incubation in aerobic conditions, characteristic colonies of *Streptomyces* are observed, and pure culture is obtained by plating a single colony on selective media.

First, morphological characterization was performed based on the morphology of *Streptomyces* colonies, air mycelium morphology, reverse side color principles after colony proliferation. Their isolation methods and the back and front mycelial images are given in Figure 1.

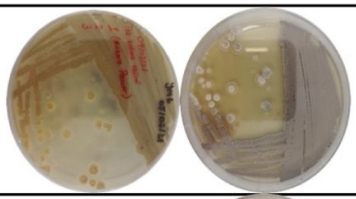
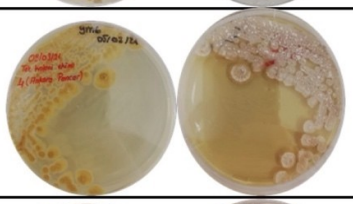
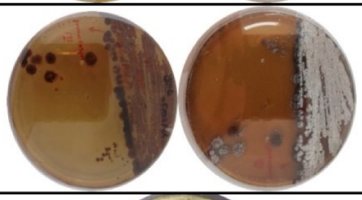
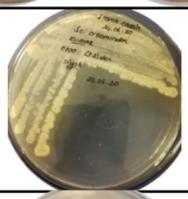
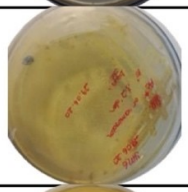
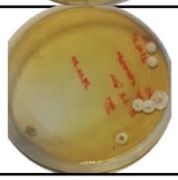
Soil Sample Area	Isolated microorganism type	Gram Staining (+/ -)	Isolation pathway	Back/front mycelial images
Ankara	*Streptomyces mutabilis*	+	**Method 1 ve 3** First inoculation from KU medium to SC medium at a dilution of 10^0, black hairy microorganism	
	Streptomyces collinus	+	**Method 3** First inoculation, white hairy microorganism from SC medium at 10^2 dilution ratio (inoculation from SC to SC)	
	Streptomyces peucetius	+	**Method 2** Black hairy microorganism by inoculation a single colony from GAM medium to MSCAM medium at a dilution ratio of 10^2.	
Konya	*Streptomyces cyaneofuscatus*	+	**Method 3** First sowing at 10^2 dilution ratio, black microorganism (inoculation from SCAM to SCAM)	
	Streptomyces albogriseolus	+	**Method 1** First inoculation from AC medium at a dilution of 10^0, white microorganism (inoculation from AC to YMG inoculation)	
	Streptomyces griseoflavus	+	**Method 1** First inoculation from KU at a dilution of 10^0, yellow microorganism (inoculation from KU to YMG)	

Figure 1: The isolated microorganism species and the isolation pathway.

S. mutabilis, S. albogriseolus and *S. griseflavous* are isolated with KU and AC agar medium with Method 1. Although they are isolated with the same method, they have different aerial and reverse side mycelia color. The only *Streptomyces* species that is formed in the GAM medium with Method 2 is *S. peucetius* which is unlike the others in terms of aerial and reverse side mycelia color and soluble pigment color. *S. mutabilis, S. collinus* and *S. cyaneofuscatus are the Streptomyces* species that are isolated with Method 3.

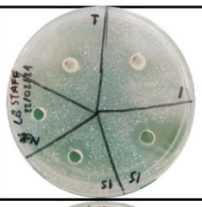
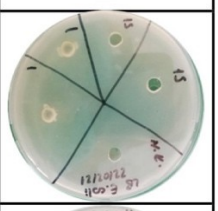
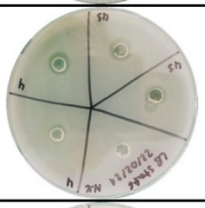
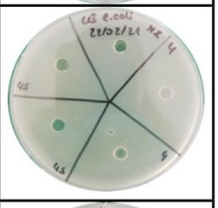
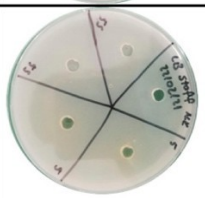
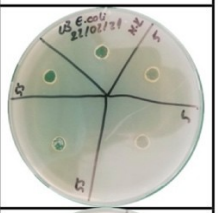
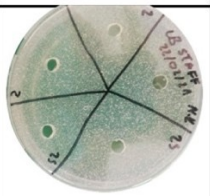
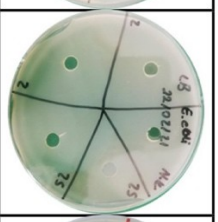
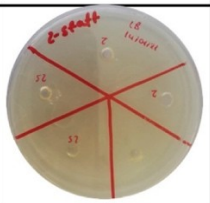
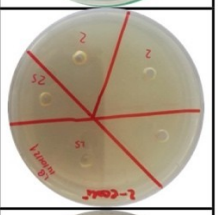
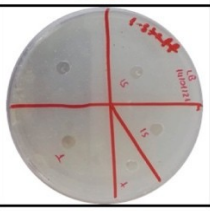
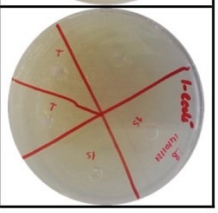
Soil Sample Area	Isolated microorganism type	Well Diffusion Method (for *Staph* and *E.coli*)	*Staph*	*E. coli*
Ankara	*Streptomyces mutabilis*	Effective on *Staph*		
	Streptomyces collinus	Effective on *Staph*		
	Streptomyces peucetius	Not effective		
Konya	*Streptomyces cyaneofuscatus*	Not effective		
	Streptomyces albogriseolus	Not effective		
	Streptomyces griseoflavus	Not effective		

Figure 2: Antimicrobial activity of isolated microorganism species on two different pathogenic microorganisms.

In Figure 2, the activities of *Streptomyces* species isolated from soils in Ankara and Konya regions on *Staph* and *E. coli* pathogens by well diffusion method are given. It was concluded that none of the microorganisms were effective on *E. coli* for Ankara soil. However, *S. mutabilis* shows the most prominent activity for the *Staph* pathogen, as well as *S. collinius*. As can be seen from the petri images of Konya soil, it was concluded that none of the microorganisms were effective on *E. coli* and *Staph*.

DNA sequence analysis results and DNA sequences are given in Figure 3 and PCR gel image of microorganisms isolated from the regions Ankara and Konya are given in Figures 4 and 5.

Soil Sample Area	Isolated species	Ratio	DNA sequence
Ankara	*Streptomyces mutabilis*	99.88%	TAAGGCGGCGGGGCCTTACCATGCAAGTCGAACGATGAACCACCTTCGGGTGGGGATTAGTGGCGAAC GGGTGAGTAGCACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACGG GATACTGACCCTCGCAGGCATCTGCGAGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTA TCAGCTAGTTGGTAGAGCGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACC GGCCACACTGGGACTGAGACACGACCCANNNTCTAATACGGGAGGCAGCAGTGGGGAATATTGCACA ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCA GCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCAC GTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAG GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGGCCAAACTGACCCTGAAGAGCAAAANCGTGGGGAACGAACAGGATTTAAATCCCC TGGAAGCCCCCCCCCCNAAACGGGGGGGCCCAAGGGTGGGGGGACATTTTCCANTTNTCCGGGCCCCCC CTAACCCTTTAAATGCCCCCCCCGGGGGAGACNNCCCCCAGGGTTAAAACTCAAGGAATTNTGGGGA
	Streptomyces collinus	99.42%	AAATTANNNTGGGACTTCCCAGGGCGGGGGCACTTAATGCGTTAGCTGCGGCACGGACAACGTGGAAT GTTGCCCACACCTAGTGCCCACCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCA CGCTTTCGCTCCTCAGCGTCAGTATCGGCCCAGAGATCCGCCTTCGCCACCGGTGTTCCTCCTGATATC TGCGCATTTCACCGCTACACCAGGAATTCCGATCTCCCCTACCGAACTCTAGCCTGCCCGTATCGACTG CAGACCCGGGGTTAAGCCCCGGGCTTTCACAACCGACGTGACAAGCCGCCTACGAGCTCTTTACGCCC AATAATTCCGGACAACGCTCGCGCCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGCGCTTC TTCTGCAGGTACCGTCACTTTCGCTTCTTCCCTGCTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCC TCACGCGGCGTCGCTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAG TCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCGCCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGT GAGCCACTACCTCACCAACAAGCTGATAGGCCGCGGGCTCATCCTGCACCGCCGGAGCTTTCGAACCG TCTGGATGCCCAGACGGATCAGTATCCGGTATTAGACCCCGTTTCCAGGGCTTGTCCCAGAGTGCAGG GCAGATTGCCCACGTGTTACTCACCCGTTCGCCACTAATCCCCACCGAAGTGGTTCATCGTTCGACTTG CATGTGTTAAGCACGCCGCCAGCGTTCGTCCTGAGCCAGGATCCAAACTCTAAAAA
	Streptomyces peucetius	99.65%	TACCGGGGCGNCTCCCAAGGGCGGGGANCTTAATGCGTTAGCTGCGGCACCGACGACGTGGAATGTCG CCAACACCTAGTTCCCAACGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCT TTCGCTCCTCAGCGTCAGTAATGGCCCAGAGATCCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCG CATTTCACCGCTACACCAGGAATTCCGATCTCCCCTACCACACTCTAGCCTGCCCGTATCGAATGCAGA CCCGGGGTTAAGCCCCGGGCTTTCACATCCGACGCGACAAGCCGCCTACGAGCTCTTTACGCCCAATA ATTCCGGACAACGCTTGCGCCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGCGCTTCTTCT GCAGGTACCGTCACTTTCGCTTCTTCCCTGCTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCAC GCGGCGTCGCTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTG GGCCGTGTCTCAGTCCCAGTGTGGCCGGTCGCCCTCTCAGGCCG CTACCCGTCGTCGCCTTGGTAGGCCATCACCCCACCAACAAGCTGATAGGCCGCGGGCTCATCCTTCAC CGCCGGAGCTTTCAACCGCAGACCATGCGATCCGCAGTGTTATCCGGTATTAGACCCCGTTTCCAGGGC TTGTCCCAGAGTGAAGGGCAGATTGCCCACGTGTTACTCACCCGTTCGCCACTAATCCCCTCCCGAAGG AGGTTCATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCGTCCTGAGCCAGGATCAAACTCT AAA
Konya	*Streptomyces cyaneofuscatus*	99.76%	CCAGGGCGCGGCTTAACNATGCAAGTCGAACGATGAAGCCTTTCGGGGTGGATTAGTGGCGAACGGGT GAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATA ACACTCTGTCCCGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGC TTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAA AGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAG AAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACG TAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGT GAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCG GAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCT GGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCA CGCCGTAAACGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAA GTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAAATTGACG
	Streptomyces albogriseolus	99.88%	ACCGGGGGGGGCTTACCATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGT GAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATA CTGACCCGCTTGGGCATCCAAGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAG CTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCAC ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGA AAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAA GAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTG TGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATC GGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTC TGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC ACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTA AGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACG
	Streptomyces griseoflavus	98.95%	CCCCGGCGGGNCTTACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGT GAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATA CTGATCCGCCTGGGCATCCTGGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAG CTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCAC ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGA AAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAA GAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGATG TGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCAGGCTAGAGTGCGGTAGGGGAGATC GGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTC TGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC ACGCCGTAAACGGTGGGAACTAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTA AGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGG

Figure 3: DNA sequence analysis results of isolated microorganism species.

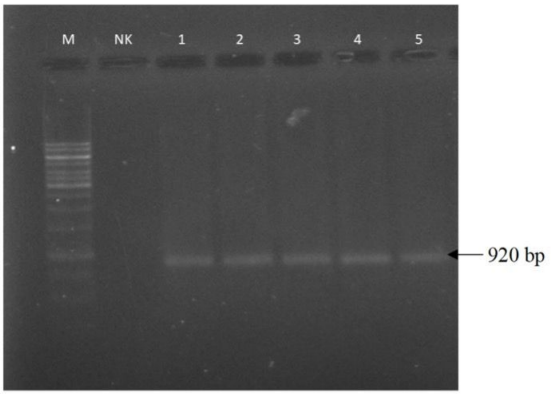


Figure 4. PCR gel image of microorganisms isolated from Ankara soil. M: 1 kb Marker. NC: Negative control, 1- *S. mutabilis*, 4- *S. collinus*, 5- *S. peucetius.*

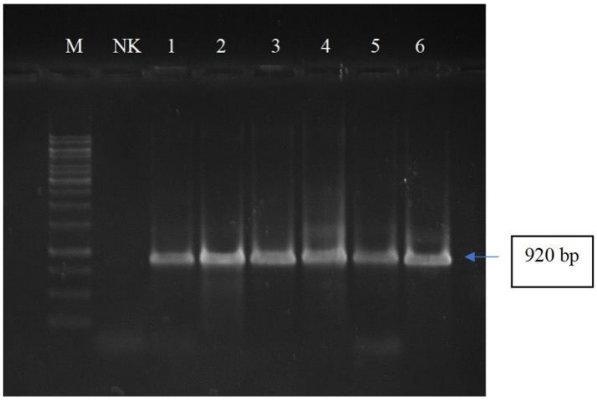


Figure 5. PCR gel image of microorganisms isolated from Konya soil. M: 1kb Marker. NC: Negative control, 1-*S. cyaneofuscatus*, 2- *S. albogriseolus*, 6- *S. griseoflavus*

Chemical, molecular, and taxonomic properties of the genus *Streptomyces* have been revealed by several studies. Siddique et al. studied *S. avermitilis* isolation from different soil samples of of Pakistan. Different media compositions were applied for the screening of pure *Streptomyces* species and antibacterial and antifungal activities of various isolates were studied (22). Arifuzzaman et al. investigated screening *Actionmycetes* from Sundarbans soil for antibacterial compounds against some gram-negative pathogenic bacteria. For this purpose, a GAM isolation medium was used and it was concluded that Karanjal region of Sundarbans is rich in *Actinomyces* (23). Kumar et al. studied the isolation of *Actinomycetes* from the soil samples of the wasteland and garden of Ghaziabad and assessed their anti-bacterial properties. The SCAM medium was used according to that purpose and fifteen isolates of *Actinomycetes* showed activity against bacteria (24). In the present study six isolates, *S. mutabilis, S. collinus, S. peucetius, S. cyaneofuscatus, S. albogriseolus* and *S. griseoflavus,* are compared with the general characteristics of the *Streptomyces* species with 3 different methods specific to *Streptomyces* species. Air, reverse side mycelium colors, and antimicrobial activities are determined.

CONCLUSION

In this study, six *Streptomyces* species are isolated from soil samples collected randomly from Ankara and Konya regions. Studies showed that the regions of Ankara and Konya are rich in *Streptomyces* species and Methods 1-3 can be used for the isolation of *Streptomyces* species. If the specific *Streptomyces* species are to be isolated from soil, then the isolation medium in Method 1-3 must be chosen carefully to reach that specific *Streptomyces* species. Further study is in progress to see and improve antibiotics production of isolated *Streptomyces* species with fermentation using optimization methods.

CONFLICT OF INTEREST

There is no conflict of interest.

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